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OSMOTIC CHANGES IN THE BLOOD SERUM OF THE PIKE *ESOX LUCIUS* L. DURING THE PROCESS OF ADAPTATION TO THE INCREASED SALINITY OF WATER

ZMIANY OSMOTYCZNE SUROWICY KRWI SZCZUPAKA *ESOX LUCIUS* L. W PRZEBIEGU PROCESU ADAPTACYJNEGO DO ZWIĘKSZONEJ KONCENTRACJI ZASOLENIA WODY

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Osmotic properties (osmotic pressure and concentration of Na^+, K^+ and Ca^{++}) of the blood serum of the pike *Esox lucius* L. were studied in natural environments of various salinity (Lake Dąbie – fresh water; Szczecin Bay – 0,4 – 1,2 per thousand salinity; Pomeranian Bay –5,4 – 8,6 per thousand salinity) and in synthetic sea water of a salinity of 2, 7, 12, 13, 14 and 16 per thousand.

The present-day studies on the osmoregulation of fishes, preceded by a number of earlier investigations (**Bert**, 1871; **Siedlecki**, 1903, and others), can be devided into two groups:

1. studies of the mechanisms of ionic and osmotic regulation (part of hormones, way of ionic-aqueous exchange between the organism and its environment) and,
2. determination of the range of ionic and osmotic regulation, giving attention to individual (age, sex, etc.), specific and environmental differences and to biological rhythms.

Studies on the mechanisms of ionic-osmotic regulation (**Chester Jones** et al., 1969; **Donaldson** et al., 1968; **Lam**, 1967; **Leatherland** et al., 1969; **Maetz** et al., 1961;

Pequignot, 1966, and others) ahowed that the hormones of the hypophysis, adrenal cortex, thyroid and those of renal and gill origin take part in the osmoregulation of fishes.

In fishes ionic-aqueous interchange between organism and environment occurs through the gill epithelium, intestines, kidneys and partly through the skin (Motais et al. 1969; Privolnev, 1967, and others).

Studies on ionic-osmotic regulation in fishes (among other authors Kashiwagi Masaaki et al. 1969, Coche, 1967, and Houston 1960) proved that in various fish species (regardless of the typical environment of their occurrence) the larvae and young fishes have the lowest osmoregulative capacity, which increases with individual development.

Most investigators (Gordon, 1959; Smith, 1956, and others) state that salinity tolerance is independent of sex and season and they think that a slight decrease in tolerance observed in some cases in the summer is connected with the seasonal rise of temperature of the environment.

Studies on osmoregulation fave chiefly been carried out on euryhaline and sea fishes, considerably fewer of them concern the stenchaline freshwater fishes.

Cordier et al. (1953), Callamand et al. (1951) and other investigators showed that an increase in the salinity of water causes a decrease in oxygen consumption and a remarkable loss in body weight owing to the loss of water in the organism of freshwater fishes.

Julien et al. (1957) demonstrated that the survival rate of the tench, catfish and roach in solutions of Na^+, K^+ and Ca^{++} of the same concentrations equal to 6 g NaCl per 1 was the highest at the lowest K:Ca ratic in solutions of two components and at the highest Na+K:Ca ratio in solutions of two components and at the highest Na+K:Ca ratio in solutions of three components.

Using the isotopic method in his experiments on the young pike, Schlumpberger (1966) found that the rate of exchange of 50% Na^{22} was directly proportional to the increase in water salinity. In the 11–12 per-thousand NaCl solution there occurred a rapid increase in the expulsion of the isotope and distrurbances in motion leading to the death of the fish.

From among freshwater species the pike deserves special attention as it is a valuable commercial fish, chracterized by large increases in body weight and feeding chiefly on fish species of small value.

The increase of the production of pike in the reservoirs of brackish water rich in food for this species and in the littoral zone of the Baltic Sea depends to a great extent on our knowledge of its biology and especially its osmoregulative capacity, determining its distribution and range of occurrence under variable conditions as regards salinity.
In this connection, the purpose of this study was to examine:

1. the level of osmotic pressure and concentration of sodium, potassium and calcium ions in the blood serum of the pike under natural variable conditions of salinity of environment;

2. the changes in the blood serum and in the range of osmoregulative capacity of the pike staying in sea water of various salinity, and;
3. the osmoregulative capacity of the pike in the aspect of seasonal biological rhythms.

MATERIAL AND METHOD

This investigation was carried out on 850 pike (462 males and 388 females) from Lake Dąbie, Szczecin Firth and Pomeranian Bay from September 1968 till November 1970.

Experiments were made in the natural invironment and under laboratory conditions (in synthetic sea water in aquariums).

The range of osmoregulative capacity was examined in fishes in water of a 2, 7, 12, 14 and 16 per-thousand salinity.

The changes in osmotic pressure and concentration of electrolytes in the blood serum were studied in fishes remaining in water of a 12, 13 and 14 per-thousand salinity for 2, 4, 6, 12, 24 and 48 hours.

Blood was taken from the heart by Dorson's method.

Serum was obtained about 30 minutes after the occurrence of retraction by centrifuging the blood for 15 minutes at 2,200 r.p.m.

The osmotic pressure of the serum was measured using a Fiske osmometer (made in the USA) and the results are given in milliosmoles per litre (mOs/1).

Na^+, K^+ and Ca^{++} content was determined by means of a Zeiss flicker photometer in milliequivalents per litre (mEq/1).

Standard solutions of sodium, potassium and calcium were prepared by Homolka's method (1961).

Synthetic sea water was made up according to the formula given by **Dietrich** and **Kalle** (1957), its oxygen content was determined by Winkler's method and pH was measured with an LBS-61 pH-meter.

The results obtained were worked out statistically by determining arythmetic means ($\bar{x}$), standard errors (S.E.) and coefficients of significance at the confidence level Q = 0,95 using Student's t test.

RESULTS

The study carried out in the natural environment (Fig. 1) shows that the values of parameters of the blood serum examined in the pike (osmotic pressure, concentrations of Na^+ and K^+) do not differ significantly between sexes. The values were found to be slightly higher in the autumn as compared with those obtained in the spring and to rise with increase in the salinity of environment.

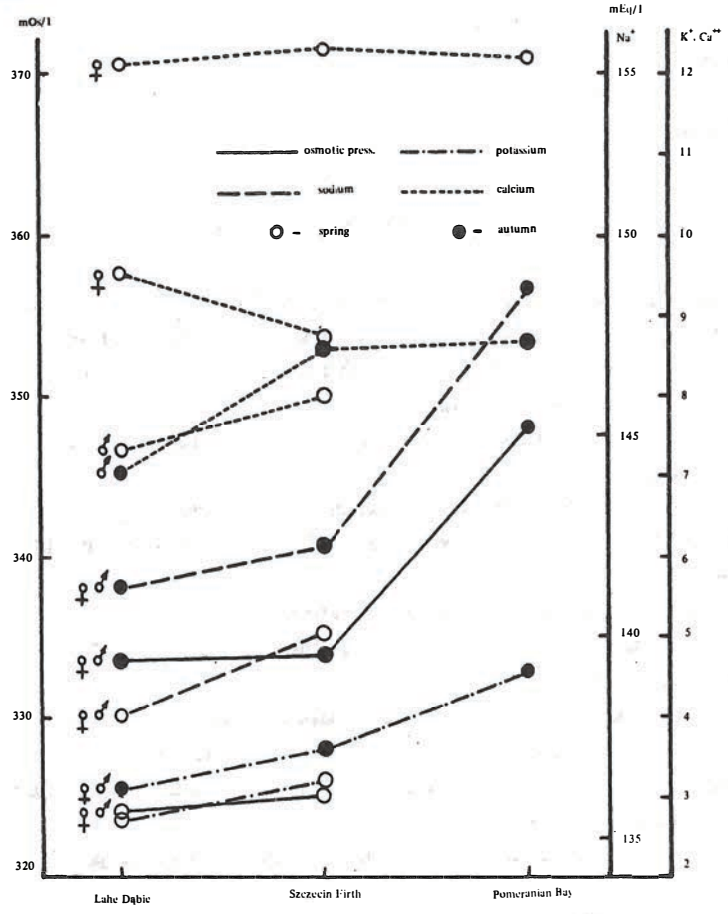

Fig. 1. Differences in osmotic pressure and Na^+, K^+ and Ca^{++} content of pike blood serum in natural environment

The concentration of serous calcium has lower values in females, rises in both sexes in the season preceding spawning and responds with no significant changes to the changes in the salinity of environment.

The results of Series I of aquarium experiments (Fig. 2) show that the transfer of fishes to sea water induces a significant increase in osmotic pressure and concentration of Na^+ and K^+ in serum. No significant changes occur in the concentration of serous calcium.

Except for signs of short-lasting excitation, the fishes placed in water the salinity of which increased to 12 per thousand showed no deviation from the normal behaviour and no changes in their responses to external stimuli.

Changes in the behaviour of fishes placed in water of 14 and 16 per-thousand salinity occurred in the following phases:

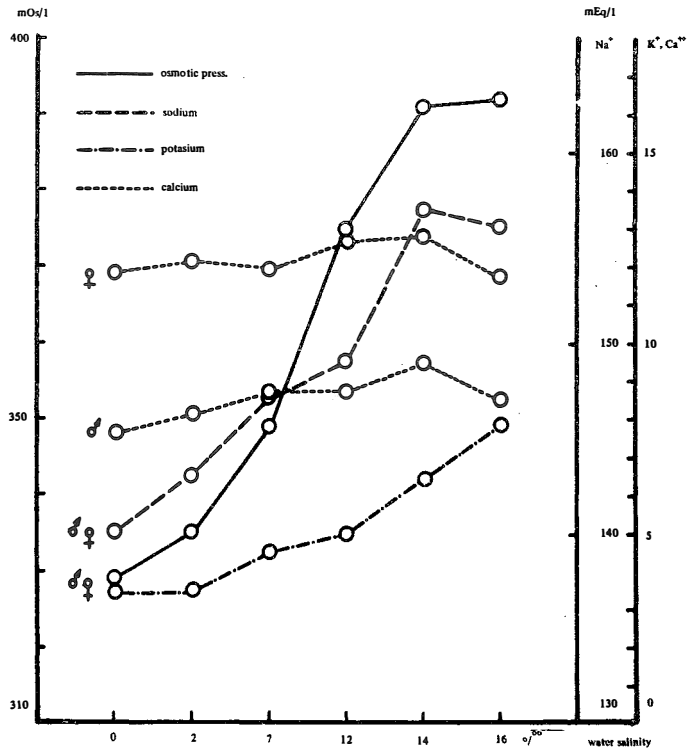

Fig. 2. Dependence of ionic-osmotic changes in pike blood serum upon increases in water salinity (autumn 1968)

1. phase of excitation – violent movements, attempts to jump out of the aquarium, accelerated respiratory rhythm, secretion of large amounts of mucus;
2. preagonal phase – subsidence of motor excitation, slowing of respiratory rhythm, temporary apnoea, decline of reaction to acoustic and mechanical stimuli;
3. agonal phase – disturbances in body equilibrium, swimming on the side, passive rising to the surface, decline of respiratory rhythm, death.

The agonal phase occurred after about 4 hours in fishes kept in water of 14 per-thousand salinity and after 2 hours at the 16 perthousand salinity.

The results of Series II of aquarium experiments (given together for 1969 and 1970) show a distinct increase in osmotic pressure and concentration of Na^+ and K^+ (maximum values after 6 hours) in the blood serum of fishes kept in water of 12 per-thousand salinity in the spring season (Fig. 3). In the further course of experiment the potassium concentration lowered. The behaviour of the fishes, after a transitory period of restlessness, was within standard limits of the species.

At the 13 per-thousand salinity (Fig. 4) the increase in osmotic pressure and concentration of sodium and potassium in the blood serum of fishes took place in a shorter time

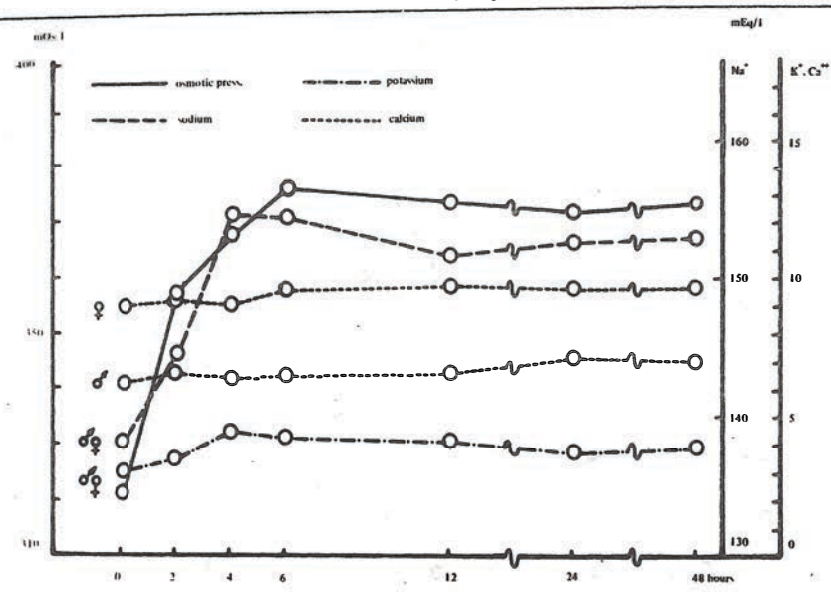

Fig. 3. Ionic-osmotic changes in pike blood serum at salinity of 12 par thousand (spring 1969, 1970)

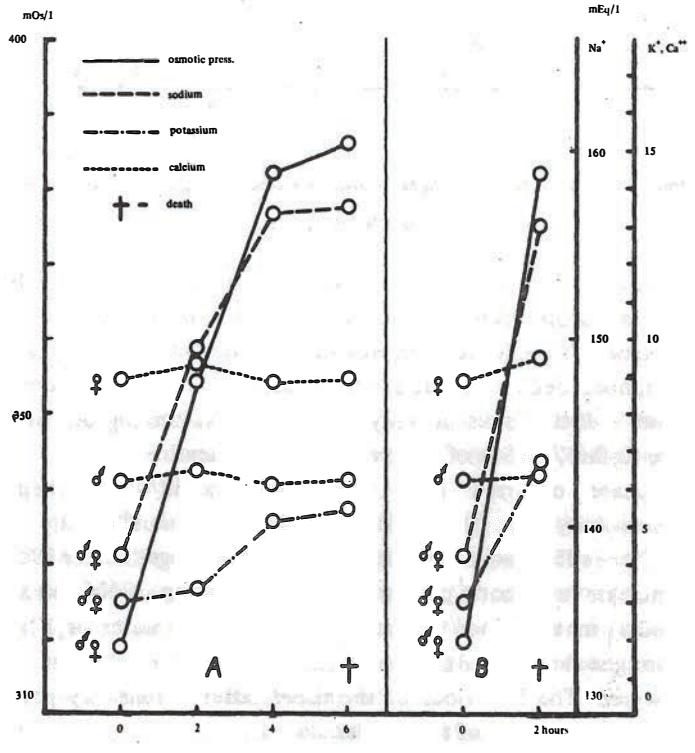

Fig. 4. Ionic-osmotic changes in pike blood serum at salinities of 13 and 14 per thousand (spring 1969, 1970). A – salinity of 13 per thousand; B – salinity 14 per thousand

and reached higher values than that at the 12 per-thousand salinity. After 5–6 hours the fishes died, the symptoms being the same as described for Series 1 of experiments.

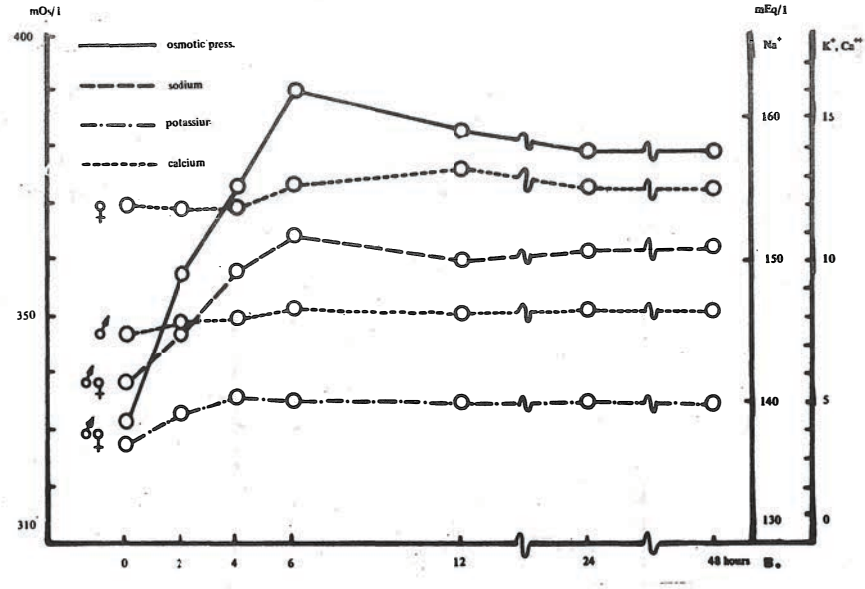

Fig. 5. Ionic-osmotic changes in pike blood serum at salinity of 12 per thousand (autumn 1969, 1970)

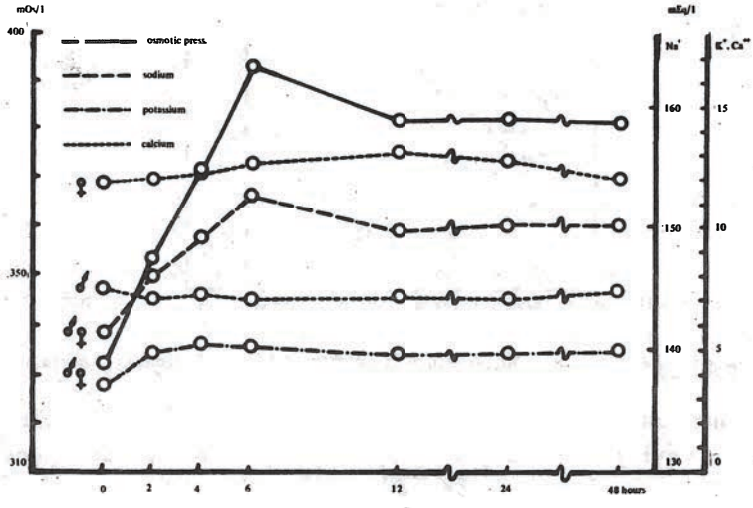

Fig. 6. Ionic-osmotic changes in pike blood serum at salinity of 13 per thousand (autumn 1969,1970)

More dramatic changes, proceeding similarly to those presented above, were found in the serum of fishes placed in water of 14 per-thousand salinity (Fig. 4b). The agonal phase occurred as early as 1,5–2,5 hours after the transfer of fishes.

In the autumn the ionic-osmotic changes in the blood serum of the pike at the 12 per-thousand salinity (Fig. 5) resembled those at the same salinity in the spring only that the potassium concentration, having reached the maximium value, kept up unchangeably at this level for the remaining course of experiment.

Changes similar to those discussed above were found in the serum at the 13 per-thousand salinity (Fig. 6). A further increase in salinity, to 14 per thousand (Fig. 7), brought about signs analogous to those observed at the 13 per-thousand salinity in the spring: rapid increase in osmotic pressure, increase in the concentration of sodium and potassium, leading to the death of fishes in about 6 hours.

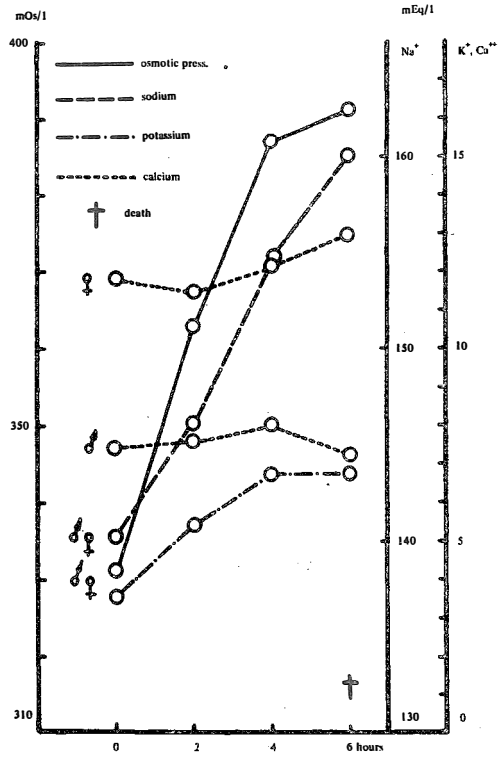

Fig. 7. Ionic-osmotic changes in pike blood serum at salinity of 14 per thousand (autumn 1969, 1970)

The concentration of serous calcium in the autumn was higher (in both sexes respectively) than in the spring and it changed slightly and independently of the changes in water salinity.

DISCUSSION

The results of studies carried out in a natural environment and under laboratory conditions presented in this paper show that the increase in osmotic pressure and concen-

tration of ions in the blood serum of the pike is directly proportional to the increase in salinity of the environment, which confirms the results obtained for other fish species (**Chan** et al., 1967; **Maetz** et al., 1968; **Mayer** et al. 1969, and other authors).

The results of my study confirm also the results published, among other investigators, by **Gordon** (1959) and **Ripplinger** et al. (1966), according to which the individual characters, such as weight, sex, etc., have no distinct influence on the ionic-osmotic changes in serum in sexually mature fishes and on their survival in the environment of increased salinity. My results, in addition, show that the time necessary for the mechanisms of osmoregulation of the pike to become adapted to an increased salinity is 6–12 hours, which is undoubtedly connected with the capability, demonstrated in this species by **Schlumpberger** (1966), to accelerate the interchange of sodium between the organism and environment remarkably.

The symptoms of the agonal phase (in a salinity of over 12 per thousand), consistent with the observations made by **Berger** (1929) and **Garrey** (1915), are most likely related to the increase of potassium in the serum and also to its effect on the functioning of the nervous system and, in consequence, on circulation and respiration.

At a lethal salinity the difficulties in gaseous interchange caused by the secretion of large amounts of mucus, coating the respiratory epithelium of the gills and probably produced as a result of the irritating action of some constituents of sea-water, seems to have been a death-accelerating factor.

Most of the authors agree that, except for a few species of salmonid fishes in which no seasonal changes in the osmotic pressure of blood serum have been found (**Gordon**, 1959), in many species the tolerance to salinity decreases in the summer (which is not always clearly connected with the increase in temperature of the environment) and also in the period of maturity of the gonads and after spawning.

Raschack's (1967, 1969) studies show that the osmotic pressure of serum of some marine fishes (especially as regards species inhabiting the zone of brakish water) increases considerably in the autumn-winter period, which fact is thought to be a form of protection against freezing and a measure to make the penetration of regions with extreme temperatures and salinities possible for these species.

Seasonal differences in the osmotic properties of serum and in the values of lethal salinity, determined in this study, seem to be chiefly associated with the spawning period, which in the pike separates the seasons examined, Spawning, which is unduobtedly a factor that changes the metabolic processes in fishes, may also be responsible for the impairment of the osmoregulative capability in this species.

CONCLUSIONS

The results obtained and discussion carried out above allow us to make the following conclusions:

1. An increase in the salinity of environment leads to the increase of osmotic pressure and sodium and potassium ion concentrations in the blood serum of the pike.

2. In natural environment the lowest osmotic pressure and sodium and potassium ion concentrations in the pike blood serum have been found in Lake Dąbie, higher in Szczecin Firth and the highest in the serum of fishes from Pomeranian Bay.
3. Under laboratory conditions it takes 6–12 hours for the osmotic pressure and sodium and potassium ion concentrations in the blood serum of pike placed in water of increased salinity to steady (adaptation).
4. The calcium ion concentration in the pike blood serum is higher in females than in males and it changes in both sexes not significantly in response to a rise in the salinity of environment.
5. The osmotic pressure and concentrations of sodium, potassium and calcium ions in the pike blood serum are lower in the spring than they are in the autumn.
6. An increase in the environment salinity to above 12 per thousand in the spring and to above 13 per thousand in the autumn causes a rapid increase in the osmotic pressure and electrolyte concentration (especially that of K^+) in the pike blood serum and leads to the death of fishes.
7. The salinity of the Baltic Sea (apart from the deepest regions, in which it exceeds 12–13 per thousand seasonally) is not the factor limiting the distribution of adult pike in this sea.

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ZMIANY OSMOTYCZNE SUROWICY KRWI SZCZUPAKA *Esox lucius* L. W PRZEBIEGU PROCESU ADAPTACYJNEGO DO ZWIĘKSZONEJ KONCENTRACJI ZASOLENIA WODY

Streszczenie

Przeprowadzono badania osmotyczności surowicy krwi szczupaka (*Esox lucius* L.) na 850 dojrzałych płciowo osobnikach pochodzących z rejonu Dolnej Odry.

W wyniku badań w środowisku naturalnym o różnym stopniu zasolenia wody (jez. Dąbie – woda słodka; Zalew Szczeciński – zasolenie 0,4–1,2‰; Zatoka Pomorska – zasolenie 5,4–8,6‰) oraz w różnych koncentracjach syntetycznej wody morskiej (2, 7, 12, 13, 14 i 16‰) stwierdzono, że wzrost zasolenia środowiska prowadzi do wzrostu ciśnienia osmotycznego, koncentracji jonów sodu i potasu w surowicy krwi oraz nie wywołuje istotnych statystycznie zmian w koncentracji surowiczego wapnia.

Stabilizacja zmian osmotycznych w surowicy krwi szczupaka przebywającego w podwyższonym zasoleniu (adaptacja) przebiega w warunkach akwaryjnych w okresie 6–12 godz.

Wzrost zasolenia środowiska powyżej wartości 12–13‰ powoduje w surowicy gwałtowny wzrost ciśnienia osmotycznego oraz koncentracji elektrolitów (szczególnie jonów K^+) i prowadzi do śmierci ryb.

Badania wykazały różnice międzypłciowe w zawartości surowiczego wapnia (na korzyść samic) oraz zwiększoną wrażliwość szczupaka na wzrost zasolenia środowiska w sezonie wiosennym (potarłowym) w porównaniu z przedtarłowym sezonem jesiennym.

ОСМОТИЧЕСКИЕ ИЗМЕНЕНИЯ СЫВОРОТКИ КРОВИ ЩУКИ ESOX LUCIUS L. ПРИ ПРОТЕКАНИИ ПРОЦЕССА АДАПТАЦИИ К ПОВЫШЕННОЙ КОНЦЕНТРАЦИИ СОЛЁНОСТИ ВОДЫ

Р е з ю м е

Проведены исследования осмотической способности сыворотки крови щуки (Esox lucius L.) на 850 половозрелых особях из района нижней Одры.

В результате исследований в естественной среде с разной солёностью воды (оз. Домбе - пресная вода; Щецинской залив - солёность 0,4- 1,2‰; Поморская бухта - солёность - 5,4-8,6‰, а также в разных концентрациях синтетической морской воды (2,7,12,13,14 и 16‰) установлено,что увеличение солёности среды приводит к росту осмотического давления, концентрации ионов натрия и калия в сыворотках крови и не вызывает статистически существенных изменений в концентрации сывороточного кальция.

Стабилизация осмотических изменений в сыворотке крови щуки, пребывающей в условиях повышенной солёности (адаптация), протекает в аквариумной среде за 6–12 часов.

Увеличение солёности среды выше значений 12–13°/оо вызывает в сыворотке резкий рост остотического давления, а также концентрации электролитов (особенно ионов K^+) и приводит к смертности рыб.

Исследования показали междуполовые различия в содержании сывороточного кальция (в пользу самок), а также повышенную чувствительность щуки к увеличению солёности среды в весеннем (посленерестовом) сезоне по сравнению с преднерестовым осенним периодом.

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